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PAPER

ANTHROPOLOGY

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Investigating Residential History Using Stable Hydrogen and Oxygen Isotopes of Human Hair and Drinking Water*

ABSTRACT: The relationship between isotopic signals in human hair and geographic region has potential forensic applications for identifying unknown individuals' place of recent residence. This study analyzes δ^2H and $\delta^{18}O$ isotopes in residential tap water and bulk hair samples from 17 volunteers representing 12 locations in Ontario, Canada. There is a strong correlation ($R^2 = 0.9$) between δ^2H and $\delta^{18}O$ values of the water samples. In contrast, the δ^2H and $\delta^{18}O$ values of the hair samples are weakly correlated ($R^2 = 0.3$), and the greater variability in the data is linked to dietary factors. This study demonstrates that the δ^2H and $\delta^{18}O$ values of hair and drinking water can be used to help identify potential place of residence in forensic cases, particularly in relation to proximity to large bodies of water such as the Great Lakes, but interpretations are complicated by the contribution of both water and diet to δ^2H and $\delta^{18}O$ values in hair.

KEYWORDS: forensic science, forensic anthropology, stable isotopes, hair, water, oxygen, hydrogen, residential history, diet

When human remains are found one of the first questions that investigators want to answer is the identity of the individual. The methods used for identification often depend on the degree of preservation of the remains; if soft tissue is still present, it may be possible to used standard identification methods (e.g., fingerprints). If, however, the remains have reached an advanced state of decomposition, these methods may not be applicable. DNA analysis, while often considered the "gold standard" in forensic evidence, is expensive and is often only useful when DNA from presumed family members is available for direct comparison.

Forensic anthropologists have developed a suite of methods to assess age-at-death, sex, ancestry, and other aspects of individual identification on badly decomposed and skeletonized human remains. Another aspect of identity in forensic cases is determining the deceased's geographic origins and recent movement, which may also aid in identification of the remains. Stable isotope analysis has been used widely in bioarcheological research to investigate past diet, geographic origins, and migration (1). The study of the stable isotopes as a geographical indicator follows the principle, "you are what you eat and drink." The body's proteins, amino acids, and fatty acids derive from food and water; therefore, the elements in a human body (such as hydrogen, oxygen, strontium, nitrogen, and carbon) reflect the isotopic composition of the diet (C, N, Sr) and water derived from a number of possible sources (H, O) (2,3). Stable isotopes are now widely used in many areas of forensic science (4,5).

This study focuses on the analysis of hydrogen and oxygen isotopes found in human hair, which are incorporated into the body from four potential sources: organic molecules from the diet, water in the diet, drinking water, and atmospheric oxygen (6–8). It is estimated that *c.* 30% of the body's hydrogen is derived directly from water consumption (6,9). Recent studies indicate that despite the variability of possible water sources, an individual's isotopic signature is, on average, similar to the isotopic signature of his/her local water source, and there is a strong relationship between the isotope ratios of tap water and annual averaged local precipitation (10,11). In general, δ^2H and $\delta^{18}O$ values are lower in precipitation at latitudes further away from the equator and away from coastal zones (12,13). This relationship permits the use of stable isotopes in forensic cases to determine the geographic origins and residential histories of unidentified human remains (14–16).

There are two stable isotopes of hydrogen, ^1H and ^2H, and three of oxygen, ^{16}O, ^{17}O, and ^{18}O, that occur naturally in water and in biological materials. The ratios of $^{18}O/^{16}O$ and $^2H/^1H$ (represented by $\delta^{18}O$ and δ^2H, respectively) change across the landscape according to geographical location, latitude, elevation, and vary along geographic gradients (1,8,12,17–19). Bowen et al. (11) note that there is a strong relationship between the isotopic values of tap water and those of annual average local precipitation, although differences do exist in some areas. The possibility of associating an individual's isotopic signature with the signature of a specific geographical region may allow for forensic applications in the identification of missing people, a particularly important tool following events such as natural

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disasters or war (20), but more regionally specific research is needed to enhance our understanding of the variation between human and hydrological isotopic values in different geographic areas. One of the key ingredients necessary for the successful use of stable isotopes in determining the recent place of residence of unidentified human remains is the availability of databases and predictive models on the spatial distribution of hydrogen and oxygen isotopes in rain and local drinking water (4). Databases are now publicly available through the Global Network of Isotopes in Precipitation (http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html), the Canadian Network for Isotopes in Precipitation (part of GNIP: http://www.science.uwaterloo.ca/~twdedwar/cnip/cniphome.html), and through Wateriso topes.org (http://wateriso.utah.edu/waterisotopes/). In addition, studies of the isotopic composition of precipitation and environmental water sources in Canada have been conducted, so baseline maps are available (21–23) and are a useful starting point for research.

This study investigates the degree to which hydrogen and oxygen isotopes can be used to discern fine geographic differences in water sources and hair of individuals who consume this water in a relatively restricted geographic area. This research focuses on a densely populated region of southern Ontario, Canada, and examines the variability in isotopic signals of residential tap water and hair samples from individuals drinking this water in 12 urban and rural locations in the province. Previous studies have examined the correlation between tap water and hair from different sources (i.e., discarded hair samples from barbershops and water samples from different areas) (8), or studied geographic variation in tap water alone (11), but to our knowledge this is the first study to analyze both hair and residential tap water samples from known donors in a restricted geographic area (i.e., southern Ontario). A study by Thompson et al. (24) collected hair samples from volunteers and barbershops in four Asian countries (China, India, Mongolia, and Pakistan), along with a subset of tap water samples, but the source of the tap water samples was not reported.

The stable isotopes of hair have been used for studies of diet and nutrition in humans, both modern and archeological (e.g., 2,3,25–28). As hair grows at a rate of *c.* 1 cm per month (15,29), a sample of hair from close to the scalp will represent the last few months of an individual's life and should yield stable isotope values that reflect the source of an individual's most recent food and water consumption. Previous research determined that there is a relatively low degree of natural variation in hair $\delta^{18}O$ values among individuals who lived in the same location for more than 6 months, although variation in $\delta^{2}H$ was reportedly slightly larger (7). This suggests that a one-time sampling of hair should provide reliable data concerning the residential history of the individual. Hair is also deemed useful in isotopic analyses because it is structurally robust, forms relatively rapidly (thus limiting the biochemical process that can change the isotopic composition of hair), and once formed (or undergoes keratinization) it does not remodel, which contributes to its utility as a potential forensic tool (7,9,30,31). In addition, through the use of known hair growth rates and models of tissue turnover (32), it is possible to track an individual's movements through testing sequential hair samples and track the movement of an individual between different geographic locations. This research examines the correlation between hydrogen and oxygen ratios in known individuals' hair samples with isotopic signals from their residential tap water in Ontario, Canada, to see whether differences can be detected in the isotopic signals of people living in different regions of the province.

Materials and Methods

Ethics approval was obtained from the Human Tissue Committee, a subcommittee of the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board. Human hair samples were collected from 17 consenting adult volunteers (three males and 14 females) who had lived in their current location for at least three continuous months. Each volunteer was instructed to take clippings of 50–100 strands of hair from the 3 cm of hair closest to the scalp and place them immediately in 25 mL screw top glass vials. Glass vials were used to store the hair samples to minimize evaporation and hydrogen exchange between the hair and moisture in the environment (24,33). Fraser et al. (34), however, found that isotopic values of ^{2}H and ^{18}O in hair samples were not significantly affected by storage method. Volunteers also provided 50 mL of water in sealed plastic vials from their tap water supply in their home. Samples were collected over a period of 2 months and were sent immediately to McMaster University where they continued to be stored in the sealed plastic vials at room temperature. The 17 volunteers represent 12 locations in Ontario and cover about 618 km of linear distance, or an area of *c.* 28,000 km^2 (Ancaster, St. Catharines, Dundas, Toronto, Port Perry, Stoney Creek, Mooretown, Brigden, Oakville, Ottawa, Lynden, and Hamilton) (Fig. 1).

To remove potential contaminants (such as particulate matter and shampoo residue), the hair samples were cleaned in a 2:1 methanol and chloroform solution for two hours, rinsed twice in distilled water, and then vacuum dried, following published protocols (3,35). Cleaning hair with organic solvents was recommended over detergents, which have been shown to damage the hair surface (2). The cleaned hair (in glass vials) and water samples were shipped to the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa (Ottawa, Ontario) for analysis. The cleaned bulk hair samples were ground on a Mixer Mill 200 (Retsch, Haan, Germany).

Three volunteers did not submit sufficient hair to provide sample material to test for both hydrogen and oxygen. Thus, of the 17 samples, 14 were tested for hydrogen, and the three smaller samples were reserved for oxygen analysis. When the samples were ground, two did not provide enough material for analysis, leaving 15 samples to be tested for oxygen. Oxygen samples were stored in a desiccator until analysis. Hydrogen samples were stored on the laboratory benchtop near the laboratory standards to ensure both samples exchanged with the environment in the same manner (6).

A Thermo Finnigan Delta^{Plus} XP isotope ratio mass spectrometer (IRMS) connected to a High Temperature Conversion/Elemental Analyzer (TC/EA) (Thermo Electron Corporation, Bremen, Germany) was used to determine the $^{2}H/^{1}H$ isotope ratios of the ground hair samples. Typically, 300 μg of the ground sample was weighed in a silver capsule. The capsules were placed in the carrousel of the Costech Zero-Blank autosampler (Costech Analytical Technologies, Valencia, CA). The pyrolysis reactor consisted of a ceramic tube (Thermo Electron Corporation, Bremen, Germany) with a glassy carbon tube insert (IVA Analytical, Düsseldorf, Germany) filled with glassy carbon chips and a graphite crucible held at 1450°C, while the postreactor column was held at 90°C. The gases were introduced to the IRMS via the Conflo 4 interface (Thermo Fisher Scientific,

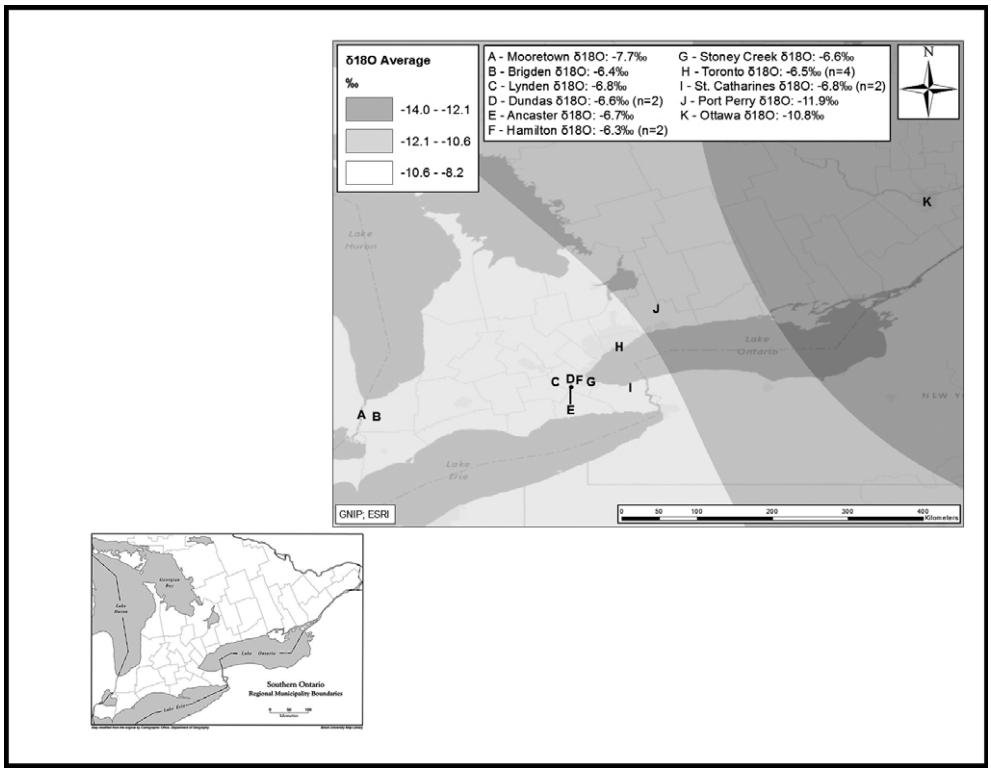

FIG. 1—*Map of southern Ontario showing sample locations and $\delta^{18}O$ values of residential tap water at each location. (Map of southern Ontario modified from: Southern Ontario-Regional Municipality Boundaries [computer file]. (no date). St. Catharines, Ontario: Brock University Map, Data & GIS Library. Available: Brock University Map, Data & GIS Library Controlled Access http://www.brocku.ca/maplibrary/maps/outline/local/Sontbase.jpg (Accessed May 20, 2015).*

Waltham, MA). Average run time for the hydrogen ratios was 300 sec.

The oxygen isotope ratios of the hair samples were determined using a PyroCube elemental analyzer (Elementar, Hanau, Germany) connected to the Thermo Finnigan Delta^{Plus} XP IRMS. Similar to the hydrogen samples, 400 μg of sample was weighed in a silver capsule, then placed in the carrousel of the autosampler (Elementar, Hanau, Germany). The pyrolysis reactor consisted of a ceramic tube with a glassy carbon tube insert (both Elementar, Hanau, Germany) filled with glassy carbon chips and a graphite crucible held at 1450°C. The resulting gasses were introduced into the IRMS via the Conflo 4 interface. Average run time for the oxygen ratios was 400 sec.

In addition to the samples, certified laboratory standards of known isotopic composition were run as calibration controls. Four internal standards for hair were run (see Table 1). In addition, three international standards for hydrogen (IAEA-NBS-30, IAEA-CH-7, IAEA-NBS-22) were run using a two-stage equilibrium protocol for each element (i.e., one set of international standards was run first, followed by ten samples from the current study; then, the second set of international standards was run, followed by the remaining study samples).

The isotopic signals of the water samples were analyzed by laser absorption spectroscopy using a Triple Isotope Water Analyzer (TIWA-45EP) (Los Gatos Research, San Jose, California). Each sample was processed by pipetting 1.0 mL into a 2 mL gas chromatography septa vial. The samples and standards were introduced without sample conversion into the instrument via a PAL HTC-xt auto injector (CTC Analytics, Zwingen, Switzerland) with a heated (*c*. 85°C) injector block (Los Gatos Research) wherein the water samples were evaporated for isotope analysis. The liquid water samples were injected into the injector block using a 1.2 μL zero dead volume syringe (Hamilton Scientific, De Pere, Wisconsin). Each sample and standard was injected ten times. Data from the first two to four injections were discarded to minimize memory effect between the samples and standards, and the other data were averaged. Three internal water standards were run at the beginning and end of each run, as well as after every 10 samples throughout the run. Three international standards for oxygen were also run (IAEA-600, IAEA-601, IAEA-602), and a further blind standard was run as an unknown and was included with each set of standards (see Table 1). All internal standards were calibrated using VSMOW, SLAP, and GISP to normalize the data.

The results are expressed as δ-values in parts per thousand (‰) relative to VSMOW (Vienna Standard Mean Ocean Water), according to the following formula:

$$\delta = \left(\frac{R(\text{sample}) - R(\text{standard})}{R(\text{standard})} \right) \times 1000$$

where R is the ratio of the heavier isotope relative to the lighter isotope, with respect to the international reference material, VSMOW, for both δ^2H and $\delta^{18}O$. The hydrogen and oxygen data were corrected using the Light Isotope Management System software (United States Geological Survey, Reston, VA). The hair results reflect nonexchangeable hydrogen values. As the samples and standards were handled the same way and are both from hair samples, the amount and value of exchange with laboratory air allows for nonexchangeable values to be presented.

Analytical precision for the water samples was ±0.5‰ for δ^2H and $\delta^{18}O$ for ±0.1‰. Analytical precision for the hair samples was ±2.0‰ for δ^2H and ±0.4‰ for $\delta^{18}O$.

Results

The $\delta^{18}O$ and δ^2H values of the tap water samples are presented on a map of southern Ontario in Figs 1 and 2, and the data are presented in Table 2. Annual average precipitation data were obtained from the Global Network of Isotopes in Precipitation (GNIP) website, and the maps were created using ArcGIS software. For the sampling sites of Toronto, Hamilton, St. Catharines, and Dundas, the average values of multiple samples are shown on the map. Fig. 3 presents δ^2H versus $\delta^{18}O$ of the tap water and hair samples. The plot in Fig. 3 demonstrates that there is a positive linear relationship between the δ^2H and $\delta^{18}O$ values of tap water (R^2 = 0.91). This line deviates slightly from the Global Meteoric Water Line (GMWL) ($\delta D = 8\ \delta^{18}O + 10$) (see 11). Most of these water samples are tap water, sourced from the Great Lakes (see Table 2). Large bodies of water are subject to evaporation, leading to an increase in $\delta^{18}O$ and δ^2H values, which is why most of the data are to the right of the GMWL (24). This also explains why both the $\delta^{18}O$ and δ^2H water data in Figs 1 and 2 are slightly higher than the average precipitation values.

TABLE 1—*List of internal standards used in this study (provided by the Hatch Lab).*

			^{18}O (‰) versus VSMOW	Deuterium (‰) versus VSMOW
Internal Standards				
AND-UK	G-737	Hair		−71.6
OTT-COL	G-736	Hair		−88.8
CAL-CAN	G-739	Hair		−106.8
CAL-SAL	G-740	Hair		−102.1
Kga-1	G-731	Kaolinite		−58.0
Kansas cellulose	C-17	Cellulose	27.9	
EIL-52	G-5267	Cellulose	20.5	
EIL-54	G-5268	Cellulose	27.5	
I-C3 + I-CH3	G-5611	Cellulose	32.0	
International Standards				
IAEA-NBS-30	G-735	Biotite		−65.7
IAEA-600	G-736	Caffeine	−3.5	
IAEA-601	C-15	Benzoic acid	23.1	
IAEA-602	C-16	Benzoic acid	71.3	
IAEA-CH-7	C-61	Polyethyl. Foil		−100.3
IAEA-NBS-22	C-62	Oil		−120.0

Oxygen

The $\delta^{18}O$ values in tap water range from −11.9‰ (Port Perry) to −5.2‰ (Toronto) (Table 2). Figure 4 presents the $\delta^{18}O$ values of 15 paired hair and residential tap water samples from different urban and rural locations in Ontario. The $\delta^{18}O$ data are in two distinct clusters. The first cluster contains samples from the western region of Ontario, including the Sarnia area (i.e., Brigden and Mooretown), the Hamilton area (i.e., Hamilton, Ancaster, Dundas, Stoney Creek), and Toronto in the upper right portion of the graph with enriched ^{18}O in both the hair and water samples. The second cluster contains the two samples from Port Perry and Ottawa in the lower left corner of the graph with values depleted in ^{18}O. The difference in the $\delta^{18}O$ values between these two regional clusters is statistically significant (Mann–Whitney *U*-test, hair: p = 0.26 and water: p = 0.22). The $\delta^{18}O$ value from Lynden (located inland and to the West of Hamilton) is slightly more negative than samples obtained from

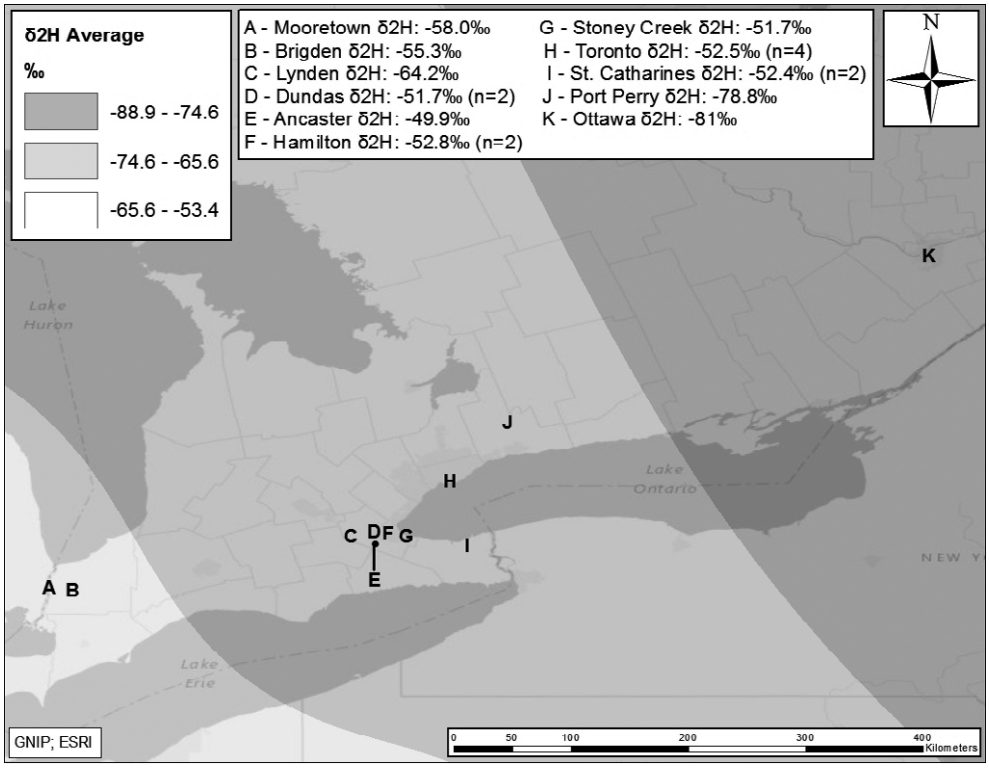

FIG. 2—*Map of southern Ontario showing sample locations and δ^2H values of residential tap water at each location.*

TABLE 2—*δ^2H and $\delta^{18}O$ values of residential tap water and hair samples from southern Ontario (bold locations indicate place names used in Figs 1 and 2).*

Sample ID (on maps)	Location	Drinking Water Source*	Water δ^2H (‰) versus SMOW	Hair δ^2H (‰) versus SMOW	Water $\delta^{18}O$ (‰) versus SMOW	Hair $\delta^{18}O$ (‰) versus SMOW
	Sarnia Area					
B	Brigden	Lake Huron	−55.3	−74.9	−6.4	−10.8
A	Mooretown	St. Clair River	−58.0	−75.9	−7.7	−10.7
K	**Ottawa**	Ottawa River	−81.0	−86.8	−10.8	−8.6
J	**Port Perry**	Groundwater	−78.8	−84.8	−11.9	−8.5
	Toronto					
H-1	Toronto	Lake Ontario	−53.6	−86.0	−6.9	−10.6
H-2	Toronto	Lake Ontario	−53.2	−76.5	−6.9	−11.1
H-3	Toronto	Lake Ontario	−49.1	−88.0	−5.2	−10.8
H-4	Toronto	Lake Ontario	−54.0	No sample	−6.9	No sample
	Hamilton Area					
E	Ancaster	Lake Ontario	−49.9	−80.7	−6.7	−9.8
D-1	Dundas	Lake Ontario	−50.4	−76.2	−6.8	−9.6
D-2	Dundas	Lake Ontario	−53.1	No sample	−6.4	−10.9
F-1	Hamilton	Lake Ontario	−52.5	−77.4	−6.8	−10.4
F-2	Hamilton	Lake Ontario	−53.0	−79.8	−5.8	−11.1
G	Stoney Creek	Lake Ontario	−51.7	−78.2	−6.6	−10.6
	St. Catharines					
I-1	St. Catharines	Lake Erie via the Welland Canal	−51.5	−86.1	−6.8	−8.6
I-2	St. Catharines	Lake Erie via the Welland Canal	−53.4	No sample	−6.7	No sample
C	**Lynden**	Groundwater	−64.2	−81.1	−6.8	−10.1

*Sources: Municipal government pages and Government of Ontario's Drinking Water Surveillance Program (https://www.ontario.ca/environment-and-energy/drinking-water-surveillance-program-dwsp-data).

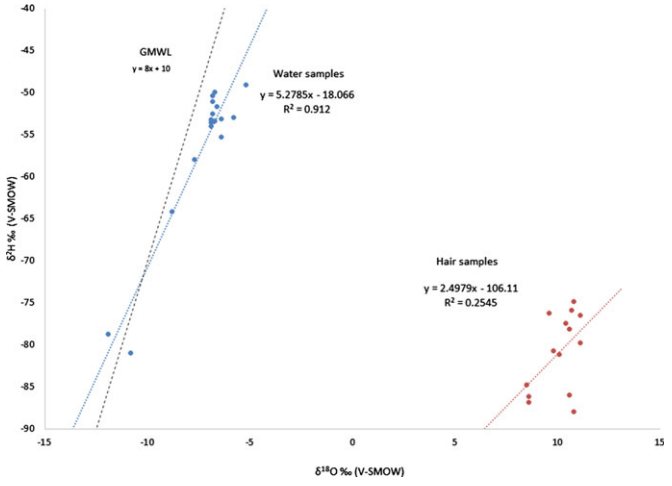

FIG. 3—*Plot of δ^2H versus $\delta^{18}O$ of Ontario tap water and hair samples. The dashed black line represents the global meteoric water line (GMWL).*

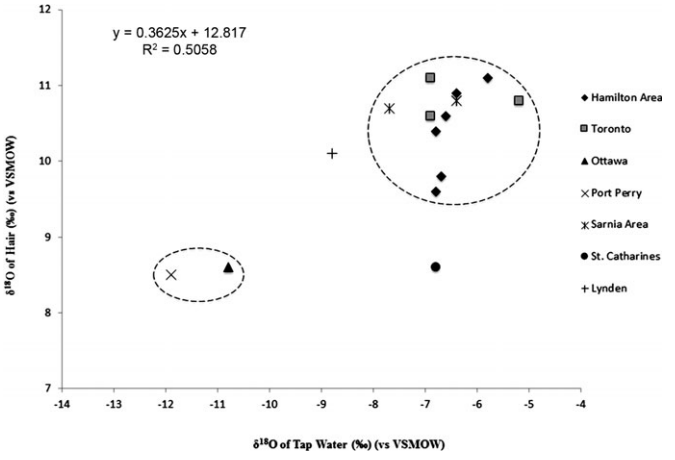

FIG. 4—*Plot of $\delta^{18}O$ values of bulk hair samples and tap water from the homes of volunteers.*

locations closer to Lake Ontario. The $\delta^{18}O$ hair and water data point for one individual from St. Catharines is also an outlier from the two main clusters with a lower $\delta^{18}O$ hair value and a $\delta^{18}O$ water value similar to other samples from the Toronto and Hamilton regions. The data cluster in relation to the sources of drinking water, with the larger group of tap water samples in Fig. 4 originating from the Great Lakes (i.e., Lake Ontario, Lake Huron, and Lake Erie), in contrast to those individuals who consumed water originating from sources further away from large bodies of water. The correlation, however, between the $\delta^{18}O$ values in the hair and water values is not strong ($R^2 = 0.51$).

Hydrogen

The δ^2H values for the tap water samples range from −49.1‰ (Toronto) to −81.0‰ (Ottawa), and the hair samples range from −74.9‰ (Brigden) to −88.0‰ (Toronto) (Table 2). These data are plotted in Fig. 5 and fall into three distinct clusters: (i) samples from the Hamilton and Sarnia areas (upper right oval), (ii) samples located further away from the Great Lakes (Ottawa and Port Perry, lower left oval), and (iii) samples that cluster in the lower right area of the chart (two from Toronto and one from St. Catharines). Similar to the $\delta^{18}O$ data, there is a pattern of higher δ^2H hair and water values from samples taken near large bodies of water (Lake Ontario and Lake Huron), and the hair and water values from Ottawa and Port Perry are isotopically distinct. There are two samples from Toronto that have surprisingly low δ^2H hair values and that cluster with the sample from St. Catharines. There is, however, no statistically significant difference between the three regional clusters identified in Fig. 5 (Kruskal–Wallis test, $p > 0.05$), and the correlation between the δ^2H values in hair and water is low ($R^2 = 0.11$), suggesting that factors other than variability in water sources are affecting these values.

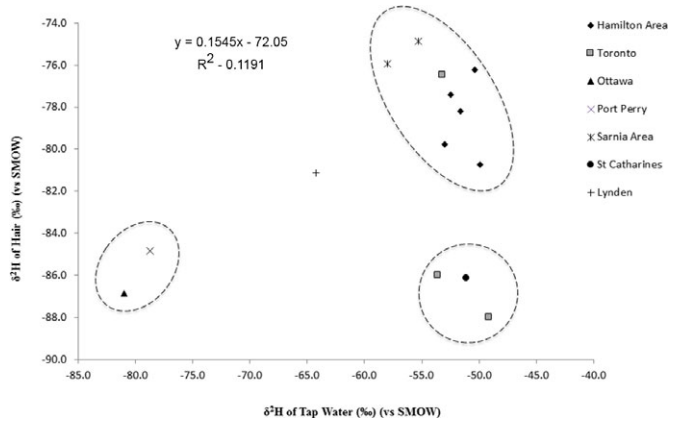

FIG. 5—*Plot of δ^2H values of bulk hair samples and tap water from the homes of volunteers.*

Discussion

The δ^2H and $\delta^{18}O$ hair data are plotted in Fig. 3, and there is a weaker correlation between these values ($R^2 = 0.26$) than the observed relationship between δ^2H and $\delta^{18}O$ values of the water samples. The low correlation of isotopic values in the hair samples is primarily due to two "outliers" (Samples H-1 and H-3, both from Toronto). If the data for these two individuals are removed, the correlation increases markedly ($R^2 = 0.70$). The low correlation between δ^2H and $\delta^{18}O$ values in the hair samples is not surprising, because oxygen and hydrogen are incorporated into hair keratin from multiple sources, including drinking water, diet, and atmospheric O_2. Oxygen enters the body as drinking water, water in food, and through uptake of atmospheric O_2 during respiration. It exits the body as urine, sweat, and respired CO_2, so body water reflects the isotopic balance between oxygen that enters and leaves the body. Hydrogen also enters the body as drinking water and food. Oxygen atoms in hair keratin are fixed primarily from water in the gut during hydrolysis of proteins, and the nonexchangeable H atoms in hair are derived from dietary amino acids and intercellular water (8,36).

Previous estimates of the amount of hydrogen in hair keratin derived from drinking water range between 27% and 42% (6,8,13,24), indicating that approximately two-thirds of hydrogen incorporated into hair comes from the diet. People living in large urban centers like Toronto can obtain proportions of their diet from a mix of both local and nonlocal sources, including food items that are imported from a wide geographical range. According to O'Brien and Wooller (13), the net effect of these global food sources is an attenuation of the expected tissue variation in hair when compared to the δ^2H and $\delta^{18}O$ values. The model developed by Ehleringer et al. (8) assumed that residents of the United States consume a "supermarket" diet that is isotopically homogeneous. However, Bowen et al. (36) demonstrated that the contribution of a local diet can have a strong influence on isotopic variation in hair, so the relative contribution of globally homogenized food sources and other, isotopically distinct, foods can have an impact on isotopic values. If there are large differences between the δ^2H and $\delta^{18}O$ values of the food and water consumed, there will be considerable variation in the relationship between the isotopic signals found in hair and that of local precipitation (37,38). Isotopic variation in the diet can, therefore, be a confounding factor in the interpretation of δ^2H and $\delta^{18}O$ data for identifying geographic origins and residential history in modern forensic cases (36).

Regarding the water results, a general pattern of higher $\delta^{18}O$ values near Lake Ontario (Hamilton area and Toronto) and Lake Huron (Sarnia area) is consistent with expected variation in oxygen isotopes in relation to distance from large bodies of water and sources of drinking water (Fig. 4). The $\delta^{18}O$ of precipitation and local drinking water varies as a function of average local air temperature, distance from the source of water vapor, and elevation (39). Many of the locations in this study (e.g., Toronto, Hamilton, Ancaster, Dundas) obtain their drinking water from Lake Ontario, as does much of South Central Ontario. Individuals living in St. Catharines obtain their drinking water from Lake Erie via the Welland Canal. Other locations (e.g., Ottawa, Port Perry, Lynden) obtain their drinking water either from groundwater or nearby river systems (Table 2). The $\delta^{18}O$ value from Lynden (located inland and to the West of Hamilton) is slightly more negative than samples obtained from locations closer to Lake Ontario, and residents there obtain their drinking water from local wells. The $\delta^{18}O$ water value of the individual from St. Catharines is consistent with $\delta^{18}O$ values from other tap water samples taken near large bodies of water (in this case Lake Erie), but this individual's hair is depleted in ^{18}O. One possible reason for this variation is that this individual consumes a specialized diet (i.e., no pork, limited chicken, and fish). As discussed above, the $\delta^{18}O$ and δ^2H signatures in hair keratin are a reflection of both water consumption and water obtained indirectly through food (9). It is possible that this individual's $\delta^{18}O$ hair value is influenced by specific dietary choices, resulting in a lower $\delta^{18}O$ value in the hair samples.

The plot of δ^2H hair versus water values (Fig. 5) shows that the δ^2H data point for the Lynden sample is situated between two data clusters, similar to the pattern observed in the plot of the $\delta^{18}O$ values. The samples from Port Perry and Ottawa produced more negative δ^2H values, which is to be expected for sites further to the North and where tap water is derived from groundwater or river sources. The δ^2H and $\delta^{18}O$ values of water samples from in and around the city of Hamilton (i.e., Dundas, Ancaster, Stoney Creek) show a high degree of consistency, with δ^2H values varying by less than 4‰ and $\delta^{18}O$ values varying by less than 1‰. These results are comparable to the small standard deviation of δ^2H and $\delta^{18}O$ values (5‰ and 1‰, respectively) observed by Ehleringer et al. (8) in the United States and indicated that households in the cities used in their study were using isotopically similar tap water. The δ^2H values of tap water for the three "outliers" (St. Catharines and two from Toronto) are comparable to other water samples from around Lake Ontario (~−50‰ to −55‰) (see Table 2), but the hair δ^2H values are lower than expected. There are a number of possible explanations for this. One of the individuals is the same St. Catharines resident who has a specialized diet, so it is likely that this individual's δ^2H hair value is also influenced by dietary factors. Similarly, the lower δ^2H hair values of the two individuals from Toronto (Samples H-1 and H-3) may also be a reflection of specific dietary choices, although their $\delta^{18}O$ values clustered with the other samples from the Toronto and Hamilton regions. Another possible explanation for the variability in the hair δ^2H values is that there was H exchange between the hair samples and moisture in the containers when the samples were collected. This, however, seems unlikely as the samples were allowed to exchange with the laboratory air prior to analysis. We therefore hypothesize that the δ^2H signals are reflecting variation in dietary sources, a potential confounding factor in the use of stable isotopes of hair to identify residential history.

Conclusions

This preliminary study of δ^2H and $\delta^{18}O$ variation in hair and water samples from southern Ontario contributes to the growing literature on the identification of residential history using stable isotope analysis of hair, which may be useful in forensic cases when dealing with unidentified human remains. This is the first study to directly compare δ^2H and $\delta^{18}O$ values in hair and drinking water samples from individuals in a densely populated region of the country, and although the sample size is small, it demonstrates that patterned variability can be detected in a relatively limited geographic area.

The $\delta^{18}O$ data presented here indicate that 14 of 15 samples tested have $\delta^{18}O$ hair and tap water values that are consistent with their location near three major lakes, and the two samples located further away from the Great Lakes (Port Perry and Ottawa), where water is obtained from groundwater or lakes, are clearly differentiated from those located near large bodies of water. The δ^2H data show a similar pattern of variation, with the individuals from locations further away from large bodies of water are isotopically differentiated from those individuals obtaining their drinking water from one of the Great Lakes, although the differences in δ^2H values between the different clusters is not statistically significant. There is considerable variability in the hair results that is likely linked to dietary factors, and it has been established in previous studies that only approximately one-third of H and O atoms in hair keratin are derived from drinking water (6,8,13,24). Further research is required to better understand the impact of dietary variability on δ^2H and $\delta^{18}O$ variation in human hair samples by collecting dietary recall information on subjects in addition to hair and water samples.

The data presented here represent the first study for Canada that uses known individuals' samples of both hair and residential drinking water. Further studies across Canada at a similar fine scale are warranted to investigate regional diversity in δ^2H and $\delta^{18}O$ values. Further research is also required to better understand the variability in hair δ^2H and $\delta^{18}O$ values in relation to dietary diversity.

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